SEASONAL VARIATIONS IN O_2-CONSUMPTION OF UCA PUGNAX

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Within the past few years studies on O_2-consumption of the fiddler crab, *Uca pugnax*, have provided descriptions of diurnal and lunar-tidal rhythms (Brown, Bennett and Webb, 1954). Webb and Brown (1958) have described the persistence of lunar-tidal rhythms of O_2-consumption for as long as three weeks in the laboratory. Both of these investigations were carried out during the summer months when these animals regularly leave their burrows at the time of low tide. Because of the marked stability exhibited by the lunar-tidal rhythm and the fact that the period of the rhythm is not one normally associated with human activity in the laboratory, it was of interest to study this rhythm at various times of year. We were particularly concerned with two aspects: (1) any seasonal variations in either the diurnal or the lunar-tidal rhythms, and (2) the degree of interdependence between the diurnal rhythm and the lunar-tidal one as indicated, for example, by the extent to which a change in one occurred simultaneously with a change in the other.

Materials and Methods

These experiments were carried out at the Marine Biological Laboratory at Woods Hole, Mass., and the animals used were collected at Chappaquoit Beach, or at Silver Beach, both on Cape Cod, Mass. During the summer months collections were made at approximately weekly intervals, the animals being picked up as they ran on the beach near the time of low tide. All of these collections were made at Chappaquoit Beach, the last one in 1959 on September 30. On January 7, 1960, and again on January 29, animals were obtained from the Supply Department of the Marine Biological Laboratory. On both of these occasions the animals were dug from their burrows at Silver Beach, which is about one mile from Chappaquoit and where low tide occurs within a few minutes of its occurrence at Chappaquoit. In the laboratory the animals were kept in large white enamel pans with a small amount of sea water which was changed daily. Only males, of the species *Uca pugnax,* ranging in weight from 2 to 4 grams were used. The animals were kept in a laboratory where they were exposed to the normal diurnal changes in illumination, and thus they experienced decreasing photoperiods until late December, and in January and February, an increasing one.

Oxygen consumption was measured by means of Brown respirometers (Brown, 1957) with the conditions the same as those described by Webb and Brown (1958). A maximum of six barostats was used at any one time. Thus the maximum num-

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ber of animals represented on any one day is 24, and the minimum is four (one barostat). Throughout most of the study three barostats (12 animals) were being used. The lever system was such that an excursion of 1.4 mm. on the record was equivalent to 1 ml. of O_2 consumed. Animals usually remained in the respirometers three or four days. Only complete days of data were used; thus the portions of records obtained between the time of setting up the respirometers and the first midnight were excluded, as were the terminal portions from midnight of the last day of each run.

Results

Data are available for 11 semi-monthly periods from July 25. 1959, to February 18, 1960, and for two such periods from June 25 to July 25, 1960. The mean values of O_2-consumption and the ranges for the mean cycles are presented in

TABLE I

Respiratory rates and cycle ranges for U. pugnax

Calendar period	Mean rate (ml./kg./hour)	Diurnal range (% of mean rate)	Lunar range (% of mean rate)
A. 7/25– 8/8	81	40%	24%
B. 8/9 – 8/24	80	33%	26%
C. 8/24– 9/8	80	28%	31%
D. 9/8 – 9/22	70	36%	37%
E. 9/22–10/6	63	28%	48%
F. 10/11–10/25	60	43%	22%
G. 10/25–11/8	48	34%	20%
H. 11/19–12/2	43	33%	22%
I. 12/4 –12/18	44	28%	27%
J. 1/9 – 1/23	46	36%	33%
K. 2/4 – 2/18	40	31%	21%
L. 6/25– 7/9	77	33%	24%
M. 7/10– 7/25	86	31%	27%

Table I. It is evident that there occurs a marked reduction in rate of respiration throughout the autumn season. The values can be roughly separated into three categories: June 25 through September 8, when the values are highest and quite constant; September 8 through November 8, when a decreasing series is obtained; November 8 through February 18, during which time the values show little or no change and are low.

It is also clear from Table I that the range of a 15-day mean cycle bears no consistent relationship to the mean hourly rate or to the season of the year. Practically the entire gamut of fluctuations in range of the solar-day cycle is encompassed within the first three periods during which the mean rate remains essentially unchanged.

In Figure 1 is seen a series of solar-day curves, each calculated for a 15-day period, plotted as deviations from the mean for the period. The periods are those recorded in Table I. It can be seen that throughout the series the form of the solar-day curve remains relatively stable. It exhibits the highest values during the

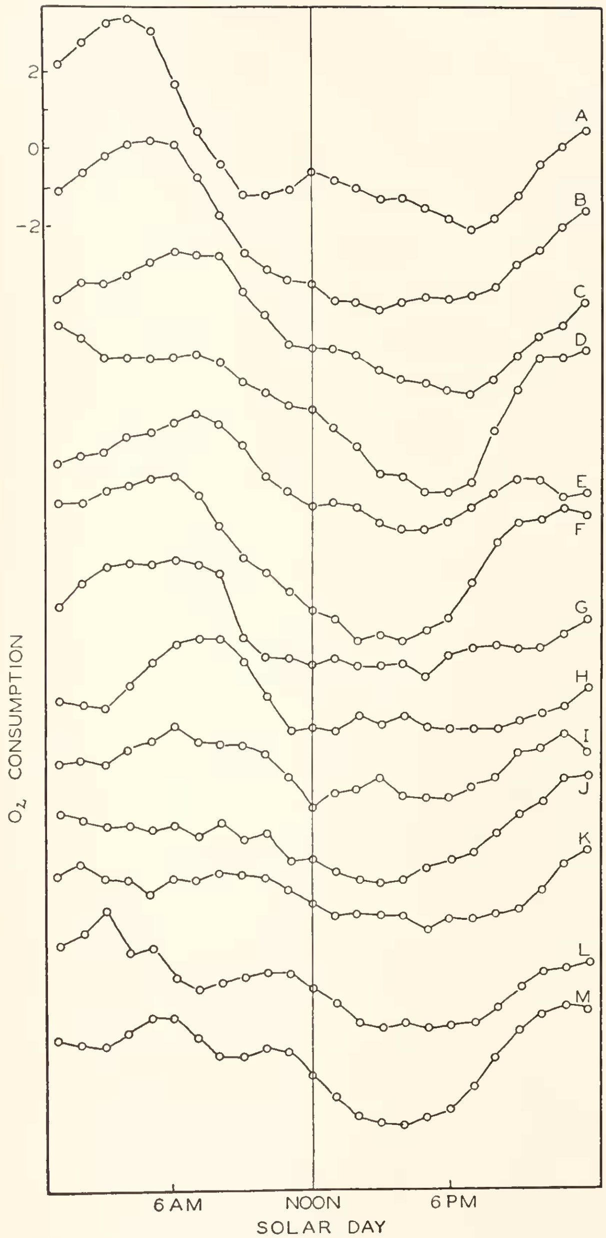

FIGURE 1. Mean diurnal variations in O_2-consumption of *Uca pugnax* for 13 15-day periods during 1959–60. The curves are for the periods appearing in Table I where each period is identified by the same letter as the corresponding curve in the Figure.

hours between 10 P.M. and noon, and the lowest values during afternoon or early evening. There are variations in position of the maximum but no obvious pattern to these variations. In Figure 2 these data are summarized in the form of four curves, one for each of the following periods: July 25 to October 25, 1959; October 25, 1959, to January 23, 1960; February 2 to 18, 1960; and June 25 to July 25, 1960. It is obvious that the absolute amplitude is reduced during the winter months but as was seen in Table I the absolute rate is decreased also. Thus when amplitude (range) is expressed as a percentage of the mean for the period, there is no consistent seasonal trend.

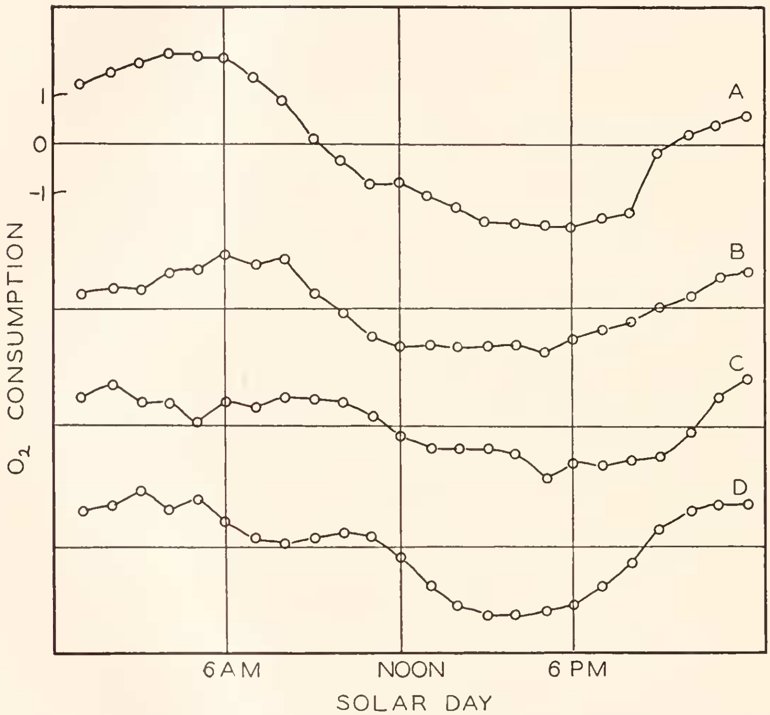

FIGURE 2. Mean diurnal variation in O_2-consumption of *Uca pugnax* for four periods throughout the year (see text for explanation).

When 15 days of data are analyzed for a lunar-day rhythm and each day is used only once, there will be present in the resulting curve a residual of the diurnal rhythm since any particular point of the solar day will have scanned only twelve hours of the lunar day. In cases where the rhythm is obviously a lunar-tidal one, with two peaks symmetrically placed in a lunar day, one can synchronize lunar zenith with lunar nadir and thus obtain complete scanning of the now approximately 12-hour day. Alternatively, one can calculate a "residual" diurnal curve and use it to make appropriate corrections on the observed 15-day lunar curve. A residual diurnal curve for each 15-day period was obtained by using the mean diurnal values which include the 15-day period for which the correction was to be

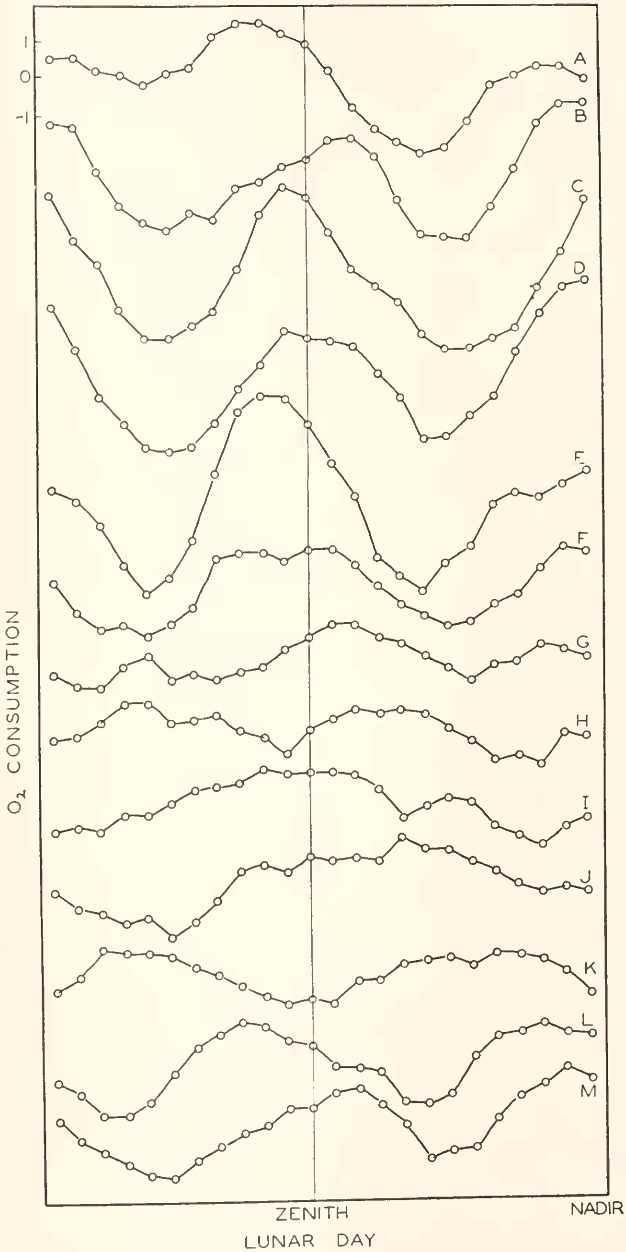

FIGURE 3. Mean lunar-day variation in O_2-consumption of *Uca pugnax* for 13 15-day periods. The letters identify the dates of the periods as found in Table I.

used. The mean diurnal values are repeated as if they represented successive days but are synchronized for lunar time and a mean value for each hour of the lunar day is thus obtained. These values then represent what would be expected if a diurnal rhythm without lunar component were analyzed for a lunar-day rhythm on a 15-day basis. The hourly values thus obtained were subtracted from the corresponding values of the observed 15-day lunar curve.

In Figure 3 is seen the series of corrected mean lunar-day curves for the same 15-day periods which have been described in Table I and Figure 1. It is seen that the first four periods (through September 22) exhibit quite symmetrically bimodal curves with maxima at or near zenith and nadir. There is a tendency for the zenith peak to alternate between an hour or two before and an hour or two after

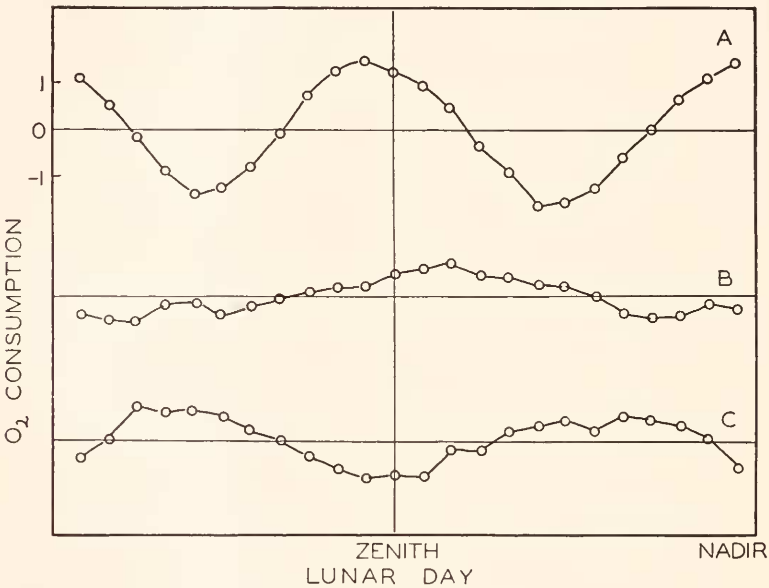

FIGURE 4. Mean lunar-day variation in O_2-consumption of *Uca pugnax* for three periods throughout the year (see text for further explanation).

zenith. During late September and early October (Curves E and F) there is some decrease in the regularity of the curves but they are still clearly bimodal and the peaks are still close to the times of zenith and nadir. Curve G (October 22 to November 6) shows a clear maximum at between one and two hours after zenith; the maximum is smoothly approached during a six-hour period on either side of it. The other twelve hours of the lunar "day" are irregular with apparent secondary maxima about three hours before lunar nadir and about five hours after it. The curves for the periods from November 19 through January 23 (Curves H, I, J) are irregular and of low absolute amplitude although, like the diurnal rhythms, the relative amplitudes show no consistent trend. These three curves on the whole seem to be unimodal in character with high values predominating near the time of lunar zenith. Curve K, Figure 3, representing the period February 2 to 18, is bimodal and in this respect resembles the "summer" curves. However, the phase relations in February are different from those observed in the summertime so that now the minima coincide with lunar zenith and nadir. The final two curves in Figure 3 are those obtained during June and July, 1960; they are bimodal with maxima near the times of zenith and nadir. Here again the tendency toward alternation of peaks around zenith is evident.

In Figure 4 are seen curves summarizing the data of Figure 3. Curve A, Figure 4 represents July 25 to October 25; Curve B is for October 25 to January

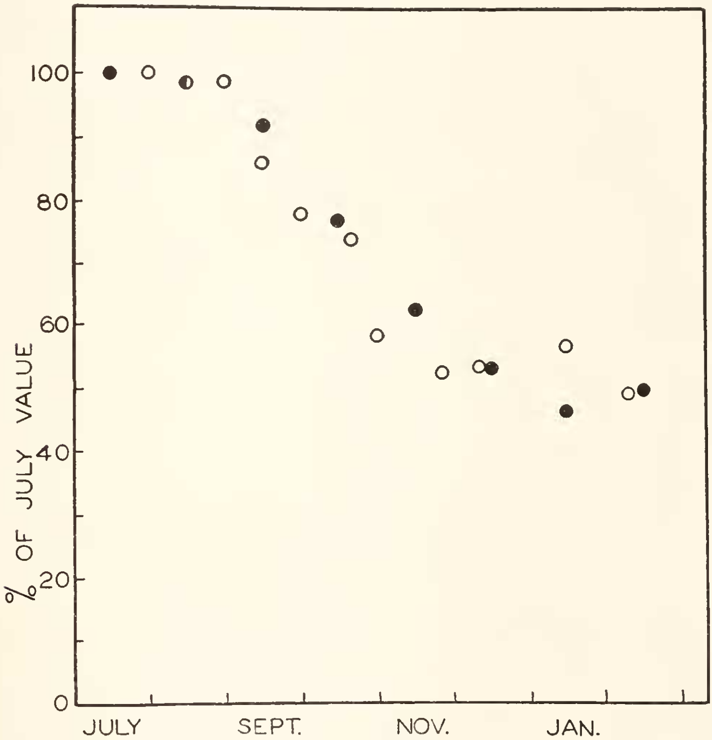

FIGURE 5. Mean monthly outdoor air temperature (solid circles) and mean rates of O_2-consumption of *Uca pugnax* (open circles). (The data on temperature were provided by Dr. Charles Packard of the Marine Biological Laboratory.)

23 and clearly reveals the unimodal nature of the curve for this period; Curve C is for February 2 to 18. From this figure it is clear that three distinct types of lunar day curves are obtained at three different times of the year.

Figure 5 contains data for the mean monthly outdoor air temperature at Woods Hole, Mass., for the months July, 1959, through February, 1960, and the mean rates of O_2-consumption calculated for each 15-day period throughout the same months. In both cases the July value is taken as 100% and the subsequent values are plotted as the appropriate per cent of that value. It is obvious that any two curves drawn through the two sets of points would be practically indistinguishable.

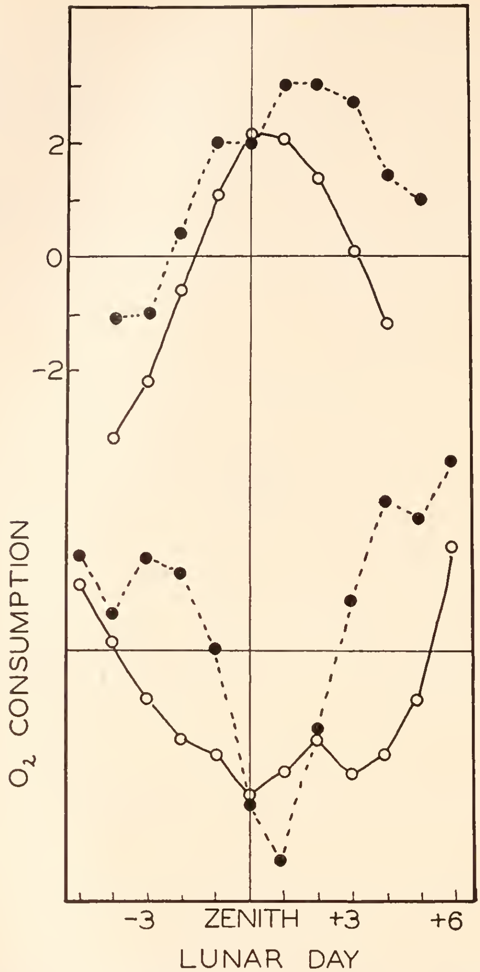

FIGURE 6. Variations in O_2-consumption of *Uca pugnax* for the hours around lunar zenith on four different days.

Figure 6 represents the values obtained, on four different days, during November and December, for the nine-hour period over lunar zenith.

Discussion

The question of the extent to which maintenance of animals in the laboratory contributes to the difference in rates observed in these studies deserves consideration. It will be recalled that collections were made on the following dates: September 30, 1959, January 7, 1960, and January 29, 1960. Therefore, at the end of the test periods October 11 to 25, January 9 to 23, and February 2 to 18, animals had been in the laboratory for 25, 16 and 20 days, respectively. Yet the rates obtained in the last two periods are considerably lower than those found for the period October 11 to 25. On the other hand, the rates observed in January and February are not significantly different from those observed in the December period. The animals used in December had been in the laboratory for 79 days by the end of the test period. Clearly it is not the length of time in the laboratory that is the major factor in the control of respiratory rates.

An interesting correlation is evident when the mean rates of O_2-consumption for successive periods are compared with mean monthly outdoor air temperature for the corresponding months, as is done in Figure 5. Since the respiratory rates were determined under constant temperature conditions, the remarkable parallel between the two series of points cannot indicate a direct cause and effect relationship. However, the obvious correlation with one factor in the physical environment (which may serve as a representative of a whole complex of annually fluctuating factors), together with the equally obvious lack of correlation with the immediate history of the animals, strongly suggests a contribution of some uncontrolled (and unrecorded) environmental factor to the apparent annual rhythm in respiratory rate.

It is realized that the results reported here are in apparent disagreement with relationships between summer and winter rates of respiration for *Uca pugnax* previously reported by Vernberg (1959) and Teal (1959). In both cases when *Uca* were tested at the same temperatures during summer and winter the rates were higher in the winter time. The animals used by both of these investigators came from different geographical areas than did those used in our experiments. Different methods of measuring O_2-consumption were used, and their methods involved much shorter test times. The maximum time for which their animals were exposed to the measurement situation would be about 8 hours and this only for the lower temperatures. In our case the first 8 to 16 hours of recording were not included in the calculations. Until some of these more obvious differences in conditions have been examined experimentally it seems unprofitable to do more than acknowledge the difference in results.

In addition to the apparent annual cycle in mean rates of O_2-consumption, the results reported here reveal variations in the form of the lunar rhythm. As was seen in Figures 3 and 4, three types of curves were encountered in going from July to February. The curve obtained in the summertime and that for February are both bimodal, one being the inverse of the other; in the intervening period a unimodal curve was obtained.

The apparently unimodal rhythm observed during the transitional period is very probably the result of averaging together for any one period curves, from single days, which show varying degrees of inversion. Evidence bearing on this question was presented in Figure 6 which shows data for the period centering on lunar zenith for four different days. It is quite clear that on two of these days distinct maxima were recorded at lunar zenith while on the other two distinct minima occurred. The relatively few days during the transitional period for which clear inversions are evident can easily be explained when it is realized that even a single record represents the sum of four animals' respiration. As an increasing proportion of the population shows an inversion of the summer pattern at lunar zenith, the chances of getting all the animals being recorded at any one time exhibiting the inverted pattern are increased. This interpretation is supported by the observation that in

February the records show inversion on more than 50% of the days while in less than 25% of the records is there a peak at lunar zenith.

Since the animals tested in December and January show similar patterns for the lunar-day rhythm, although one group has been in the laboratory for 79 days and the other for only 16, it is clear that the conditions of maintenance in the laboratory are not sufficient to explain the observed changes in the rhythm. One condition that was common to all the animals, whether in the laboratory or in the field, was the absence of illumination periods limited to the time of low tide. From early October it was not possible to collect crabs except by digging. Since they remained in their burrows they would not be exposed to all of the changes associated with low tide to which they are exposed in the summertime. These animals would presumably be in continuous darkness. The animals in the laboratory, on the other hand, would have experienced light periods longer than those to which they are normally exposed, and lacking in any tidal component. It is possible, therefore, that the absence of illumination periods associated with the tides permitted this inversion of the form of the rhythm. It is to be noted, however, that there is no evidence of a loss of precision of the period; the majority of the animals are in synchrony during February. The simplest explanation seems to be that the animals receive information concerning the time of lunar zenith and nadir. The difference between summer and winter respiratory patterns might reflect a difference in pattern of the "information" or it might represent a different sign of response on the part of the animals.

Regardless of interpretations as to cause, it is clear from the observations reported here that animals, while showing no obvious changes in the diurnal rhythm, have exhibited alterations in the lunar-day rhythm of respiration. There is no evidence of change in the period of the rhythm but there is evidence of "preferred" phase relations to lunar zenith and nadir.

Summary

1. Records of the O_2-consumption of *Uca pugnax* were obtained during the summer, autumn, and winter months and analyzed for diurnal and lunar-day rhythms.

2. An apparent annual rhythm in mean rate of respiration is reported.

3. The respiratory rate during December, January, and February was found to be approximately 50% of that during June, July, and August. During September, October, and November, a gradual decrease in rate was observed.

4. The form of the diurnal rhythm of respiratory rate showed no changes associated with time of year. Similarly, no seasonal changes in phase relations were observed.

5. The range of both diurnal and lunar-day rhythms showed fluctuations but these were not clearly associated with time of year.

6. The lunar-day rhythm was found to show changes as follows: June through September the curve was bimodal with maxima at lunar zenith and nadir; beginning in October and continuing through January the curve was unimodal with a maximum near lunar zenith; in February the curve was bimodal but with minima at lunar zenith and nadir.

7. The unimodal curve of the transitional period is interpreted as being the resultant curve of a mixed population.

8. The possible relationship of exogenous factors to these seasonal variations is discussed.

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